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Short Report

Allele frequencies for six miniSTR loci of Northwestern Chinese Han populations

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ABSTRACT

MiniSTR loci has demonstrated to be an effective approach to recover genetic information from degraded sample, due to the improved PCR efficiency of their reduced PCR product sizes. Allele frequencies and forensic parameters for the six miniSTR loci D10S1248, D2S441, D1S1677, D9S1122, D10S1435 and D17S1301 were investigated in 154 Northwestern Chinese Han populations. All loci showed a moderate degree of polymorphism with observed heterozygosity >0.6 and did not show departures from Hardy–Weinberg equilibrium for Northwestern Chinese Han populations. The accumulated powers of discrimination for the six loci were 0.999998.

1. Population

Blood samples were collected from 154 unrelated, healthy Northwestern Chinese Han individuals. The total land area of the Northwest China is about 3,110,000 km^2. There are about 90,000,000 Han population (2004) distributed in it. The ancestors of the Northwestern Chinese Han populations are Huaxia zu, they settled down there and gradually began development since about 5000 BC.

2. Extraction

DNA was extracted using the Chelex-100 protocol.[1] The quantity of recovered DNA was determined spectrophotometrically.

3. PCR

According to the previous papers[2–4] and our own study results, we chose D10S1248, D2S441, D1S1677, D9S1122, D10S1435 and D17S1301 six miniSTR loci which were suitable for Chinese populations to construct two multiplex systems (Group 1 and Group 2). Two miniSTR multiplexes PCRs were performed with the primer sets designed by Coble and Butler.[5] The primer sets listed in Table 1. Changes were made to the fluorescent dye labeled to accommodate subsequent PCR fragment analysis detection. PCR reactions were performed in a total volume of 10 μl containing 1 ng of genomic DNA, 1× Taq PCR buffer, 1.5 mM MgCl2, 200 μM of each dNTP, each primer set, and 1 U of Taq DNA polymerase (Shanghai sangon, China). One multiplex PCR mixture contained 0.2 μM primer sets of D10S1248 (fluorescent dye labeled with FAM), 0.3 μM D2S441 (HEX) and 0.5 μM D1S1677 (TAMRA). Another multiplex PCR set contained 0.3 μM primers D9S1122 (HEX), 0.7 μM D10S1435 (TAMRA) and 0.5 μM D17S1301 (FAM). Amplification was carried out using GeneAmp 9700 (Applied Biosystems) with the modified PCR condition. Pre-PCR denaturation was performed at 94 °C for 3 min followed by 28 cycles of denaturing at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s, and a final extension at 60 °C for 30 min.

4. Typing

Electrophoresis was performed using an ABI 310 Genetic Analyzer (Applied Biosystems). An appropriate matrix in this study was established with matrix standards for the four dyes 6FAM, HEX, TAMRA, and ROX. The samples for analysis were prepared by combining 1 μl of multiplex PCR product and 12 μl Hi–Di formamide containing 0.3 μl GeneScan-500 ROX size standard in a 200 μl tube followed by denaturation at 95 °C for 3 min and then 4 °C for 2 min, using a GeneAmp 9700. Samples were injected for 2 s at 15,000 V and separated at 15,000 V at 60 °C for 20 min. Data were analyzed using GeneScan 3.1.2 software. Allele designations were determined according to recommendations of the DNA Commission of the ISFG[6] by comparison with allelic ladders, which were created by molecular cloning method.

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Table 1
Primer sequences used in this study.

Loci	Primer sequence	Fragment length	Core sequence	Dye	Ref.
D10S1248	F:5′- TTAATGAATTGAACAAATGAGTGAG -3′ R:5′- GCAACTCTGGTTGTATTGTCTTCAT -3′	82–114	GGAA	FAM	[5]
D2S441	F:5′- CTGTGGCTCATCTATGAAAACTT -3′ R:5′- GAAGTGGCTGTGGTGTTATGAT -3′	80–104	CTAT	HEX	[5]
D1S1677	F:5′- TTCTGTTGGTATAGAGCAGTGTTT -3′ R:5′- GTGACAGGAAGGACGGAATG -3′	83–111	CCTT	TAMRA	[5]
D9S1122	F:5′- GGGTATTTCAAGATAACTGTAGATAGG -3′ R:5′ -GCTTCTGAAAGCTTCTAGTTTACC -3′	98–118	GATA	HEX	[5]
D10S1435	F:5′- TGTTATAATGCATTGAGTTTTATTCTG -3′ R:5′- GCCTGTCTCAAAAATAAAGAGATAGACA -3′	96–124	TATC	TAMRA	[5]
D17S1301	F:5′- AAGATGAAATTGCCATGTAAAAATA-3′ R:5′- GTGTGTATAACAAAATTCCTATGATGG -3′	106–126	AGAT	FAM	[5]

5. Quality control

Commercial DNA standard 9947 (Promega, USA), was genotyped as standard reference. A concordant study was carried out to ensure result reproducibility and accuracy. Approximately 6.5% and 9.7% of samples (10 and 15 samples) were regenotyped for the two multiplex systems, respectively. All genotype results were in full concordance.

6. Results

Allele frequency and forensic parameters summarized in Table 2.The genotyping results of one degraded blood sample (degraded outdoor for 8 weeks) in Fig. 1.

7. Analysis of data

Forensic statistical parameters were performed using the software PowerStatsV12 spreadsheet (http://www.promega.com/geneticidtools/powerststs/).[7] Hardy–Weinberg equilibrium was determined by an exact test using the GenAlEx6 software package.[8]

8. Access to data

Through e-mail from corresponding author.

9. Other remarks

The observed allele sizes ranged between 80 and 138 bp in our system. The genotype frequency distributions in the six miniSTR loci showed no deviations from the Hardy–Weinberg equilibrium by an exact test. Heterozygosity values were all greater than 0.6. The power of discrimination ranged from 0.869 (D1S1677) to 0.915 (D10S1435), whereas the power of exclusion ranged from 0.372 (D9S1122) to 0.585 (D10S1435). The expected heterozygosity, power of discrimination, polymorphism information content, and typical paternity index of D10S1435 were highest among the loci. The accumulated power of discrimination and power of exclusion for the six loci were 0.999998 and 0.978335, respectively.

In conclusion, a Chinese population database has been established for the six miniSTR systems studied. These systems have been shown to be useful tool for personal identification.

Table 2
Observed allele frequencies and forensic efficiency parameters for the six miniSTR loci in Northwestern Chinese Han population.

Allele	Group 1			Group 2		
	D10S1248	D2S441	D1S1677	D9S1122	D10S1435	D17S1301
7						0.036
8	0.003				0.052	0.146
9		0.003		0.045		0.325
10	0.058	0.276	0.013	0.175	0.068	0.325
11	0.328	0.344		0.344	0.276	0.140
12	0.276	0.205	0.036	0.344	0.302	0.029
13	0.247	0.004	0.169	0.084	0.179	
14	0.062	0.120	0.461	0.006	0.107	
15	0.019	0.006	0.240		0.016	
16	0.006		0.071			
17			0.010			
N	154	154	154	154	154	154
MP	0.117	0.106	0.131	0.119	0.085	0.102
PD	0.883	0.894	0.869	0.881	0.915	0.898
PE	0.527	0.482	0.382	0.372	0.585	0.451
PIC	0.710	0.700	0.650	0.680	0.750	0.700
TPI	2.080	1.880	1.510	1.480	2.410	1.750
p	0.365	0.522	0.808	0.692	0.529	0.510

MP: matching probability; PD: power of discrimination; PE:power of exclusion; PIC: polymorphism information content; TPI typical paternity index; *p*: values of the exact tests for Hardy–Weinberg equilibrium.

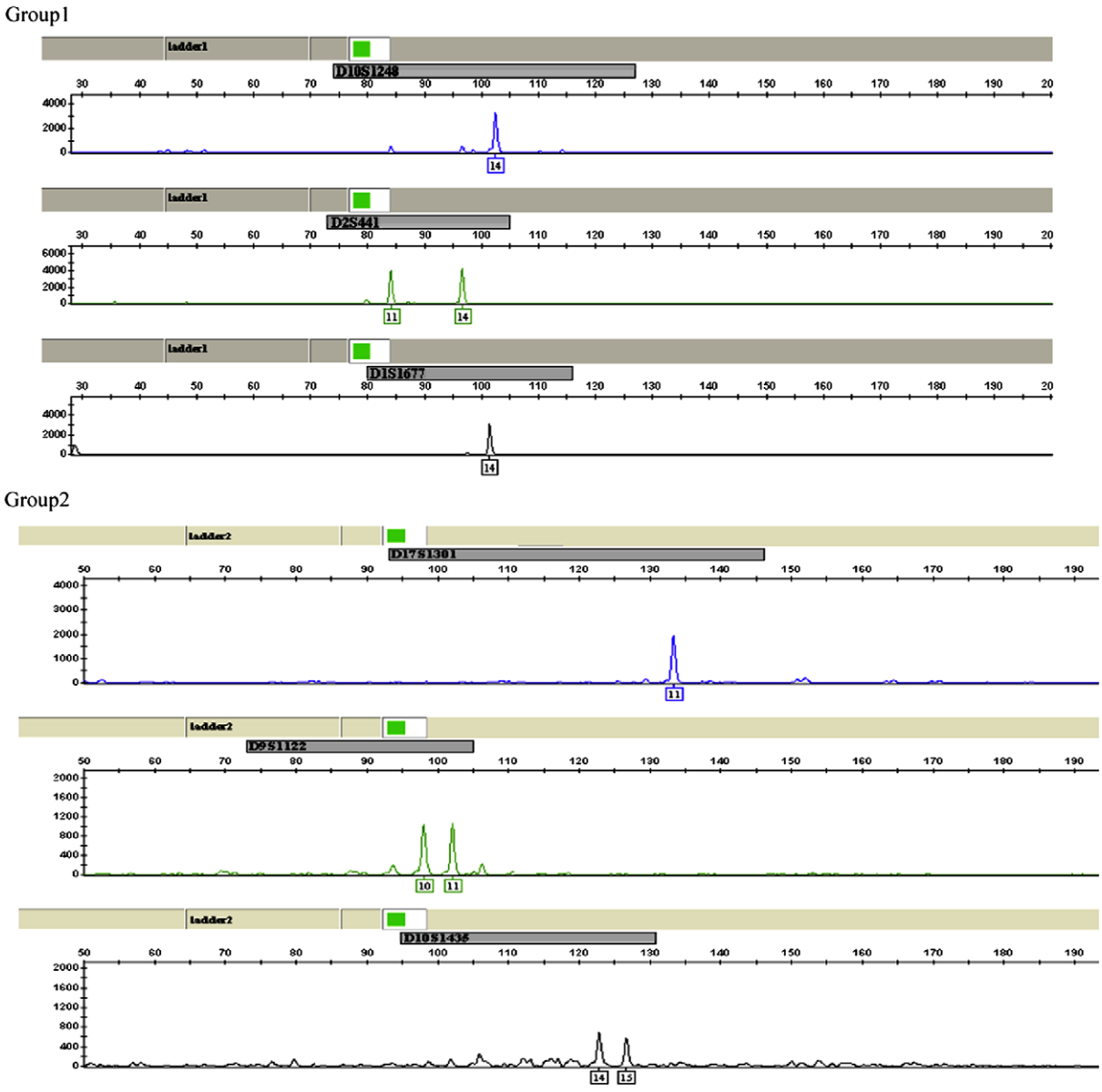

Fig. 1. Electropherogram of DNA typing result of one degraded blood sample using the present multiplex system for assay with six minSTR loci.

Conflict of Interest

We declare that we have no conflict of interest.

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